

Engineering Polyketide Synthases to Generate Lightly Branched Biofuels

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Project Goals: This project seeks to harness the biosynthetic capacities of polyketide synthases to generate lightly branched biofuels that will serve as excellent replacements for petrochemically-derived gasoline and diesel fuels. Selective methyl branching has a strong impact on the melting point and other physical properties of biofuel candidates, but can be challenging to install through other biosynthetic platforms, such as terpene synthases and fatty acid synthases. Thus, this work seeks to capitalize on the unique biosynthetic logic of polyketide synthases towards this end.

The biosynthetic logic of polyketide synthases (PKSs) has traditionally been harnessed to generate a diverse range of bioactive natural products. However, due to the ability to precisely tailor molecular structure through this biosynthetic logic, PKSs have great potential for synthetic biology applications in other areas, which have been largely underexplored. PKSs are particularly attractive candidates to generate fuels with optimal physical properties (such as lowered melting points) due to their ability to form selectively branched chemical architectures. This selective branching presents a stark advantage when compared to other commonly used biofuel platforms, such as fatty acid synthases. Current efforts in this area seeks to apply engineered PKSs to generate polyketide metabolites as petrochemical replacements for both compression ignition (diesel) and spark ignition (gasoline) fuels.

Specific engineering targets include a *de novo* strategy to generate a loading didomain that will prime a module in the middle of the borrelidin synthase. This borrelidin synthase has iterative activity, which is unusual for bacterial type I PKSs. Through a domain swap of mixed selectivity for branched and unbranched extender units, a series of compounds can be generated that could be excellent diesel replacement fuels or fuel-blends. To generate gasoline replacement fuels, an engineered version of the lipomycin PKS is being developed, which accepts small, lipophilic branched chain acyl-CoA starter units. The significant protein engineering efforts that have been undertaken to design these pathways have led to new fundamental insights regarding structural features and domain boundaries within PKSs.

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